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Drug discovery and the BBB

Measuring blood–brain barrier penetration using the NeuroCart, a CNS test battery

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To systematically study the pharmacodynamics of a CNS drug early in the development process, we developed and validated a battery of drug-sensitive CNS tests, which we call NeuroCart. Using this test battery, data-intensive phase I studies in healthy subjects can be performed to demonstrate the specific, time- and dose-dependent, neurophysiological and/or neuropsychological effects of a compound, thereby confirming whether the test compound reaches its intended target in the CNS – or does not reach its intended target. We use this test battery to demonstrate that a compound passes the blood–brain barrier.

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Introduction

To systematically study the pharmacodynamics of a CNS drug early in the development process, we developed and validated a battery of drug-sensitive CNS tests, which we call NeuroCart. Using this test battery, data-intensive phase 1 studies in healthy subjects can be performed to demonstrate the specific, time- and dose-dependent, neurophysiological and/or neuropsychological effects of a compound, thereby confirming whether the test compound reaches its intended target in the CNS – or does not reach its intended target. We use this test battery to demonstrate that a compound passes the blood–brain barrier. The battery can also be used to compare the profiles of CNS active drugs with respect to their effects on the range of tests in the NeuroCart, and allows comparison of new compounds with an innovative mechanism of action to established drugs that were previously profiled.

Whether a newly developed drug ultimately reaches the market depends largely on its targeted therapeutic area. Drugs that target the CNS generally have the lowest likelihood of reaching the market due to a variety of factors, including the complexity of the target organ (the brain), the high probability of causing CNS side effects, and the requirement of the CNS drug to cross the blood–brain barrier (BBB) [1]. Among these factors, it is fair to say that BBB penetration is one of the most significant factor in terms of restricting the development of new CNS drugs [2]. Many CNS drugs failed to reach the market because of their inability to cross the BBB; however, the worst-case scenario occurs when this is discovered only at a very late stage in the drug's development, after years of research and huge sums have been invested. For example, the gamma secretase modulator flurbiprofen (tarenflurbil) was being developed for Alzheimer's disease, but development was terminated after a phase III study failed. Only then was it determined that the drug's mechanism of action was ineffective at putative therapeutic concentrations because it did not cross the BBB [3]. Another example is Gavestinel (GV150526), a glycine antagonist that targets the *N*-methyl-D-aspartate

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receptor and was being developed for the treatment of stroke. Although several phase III clinical trials were performed, the drug failed, most likely because it did not cross the BBB [1].

Failure in phase III of a drug's development can be extremely expensive and frustrating; therefore, pharmaceutical companies have an urgent need for predicting whether a drug is likely to pass phase III testing as early as possible in the development process. In 1998, the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) established a guideline regarding the general aspects of clinical trials; the guideline states that 'The essence of rational drug development is to ask important questions and answer them with appropriate studies' [4]. Consistent with this goal, we developed a question-based system to design a structured approach for evaluating new medicines. Question-based drug development uses a logical progression of questions [5]. In this process, the first question is whether a new drug reaches its intended site of action. In the case of a CNS drug, this translates to the following question: 'Does this compound cross the BBB?'.

There are several ways to test whether a compound crosses the BBB. For example, microdialysis, autoradiography, and even whole-brain homogenization can be used in animal models. Because these methods are not feasible in human subjects, clinical trials use other techniques such as positron emission tomography (PET), single-photon emission computerized tomography (SPECT) and cerebrospinal fluid (CSF) sampling to indirectly measure BBB penetration.

Using PET, researchers can measure the localization of the radiolabeled drug in the brain; an improved technique measures displacement of a validated PET tracer from a receptor, which can provide direct evidence of both BBB penetration and binding to the relevant receptor. This approach requires either a validated PET tracer for a specific receptor (which is often not available in the case of a novel drug) or development of a radiolabeled drug (which requires changing the molecule's structure and the need for new preclinical toxicology studies before the human PET study can be performed). Moreover, PET studies (i) cannot be combined with first-in-human drug testing, (ii) are expensive to perform, and (iii) it may not be straightforward to determine the optimal binding range for compounds with complex or new action mechanisms [6]. SPECT is a less expensive alternative to PET, but it is also more difficult to quantify reliably enough for predictions of drug concentrations in the CNS [7]. Lastly, although CSF sampling provides only indirect evidence of BBB penetration (as the blood-CSF barrier is not identical to the BBB), it is generally believed that a compound that reaches the CSF will also – at least to some extent – reach the brain. Unfortunately, however, obtaining a CSF sample by lumbar puncture or spinal catheterization can be burdensome to some subjects, causing post-puncture headache in 5–50% of cases, and the test cannot definitively demonstrate that pharmacologically active levels of the compound reach the target tissue.

The penetration of a drug across the BBB is characterized by changes in brain concentrations and effects over time [6]. Consequently, establishing a concentration–effect relationship between the test compound and CNS function can be regarded as evidence of BBB penetration. A robust concentration–effect relationship is clear evidence of pharmacological activity in the CNS. Given that most modern drugs are characterized by high specificity for a single pharmacological target (for example, a specific receptor), any effects that are closely related to the plasma concentrations of such a drug are likely due to the compound's specific mechanism of action. Thus, effects that show a pharmacokinetics/pharmacodynamics (PK/PD) relationship provide clear evidence of pharmacological activity. This activity can also be expected to underlie the drug's therapeutic action, although not necessarily in the same physiological system and/or anatomical brain region. To cover the entire range of drug action, including the effects that are most closely associated with therapeutic activity, test batteries that measure a broad range of functions should be used for measuring pharmacodynamics effects throughout the CNS.

A CNS test battery: NeuroCart

Based on experience and the published literature, we selected pharmacodynamics tests that have a high sensitivity for pharmacological effects and robust reproducibility using a wide range of compounds. Indeed, the full battery of tests covers an extremely wide range of relevant domains regarding CNS function. For the purposes of neuropharmacology studies, the following six domains were defined: executive function, attention, memory, visuomotor function, motor skills, and subjective drug effects. To be included in the test battery, each test needed to be confirmed as a reliable biomarker for either intended or unintended pharmacological effects in one or more of these domains. To differentiate between a pharmacological effect on the CNS and nonspecific effects caused by peripheral activity, a clear concentration–effect relationship was required. Each biomarker (i.e., test) must also have a consistent response across studies and drugs. Finally, the biomarker must have a clear response to a therapeutic dose, and a plausible relationship must be established between the biomarker, pharmacology, and pathogenesis. Armed with these criteria, we searched the literature for optimal tests that have been used for various compounds that were known to affect CNS function. Given the large number of tests, and given the wide variation in the way the tests were performed, a formal meta-analysis was not feasible. However, we were able to identify tests that consistently showed a significant concentration-dependent effect with a given class of drug. We therefore performed several systematic literature reviews, which enabled us to evaluate in

healthy volunteers the sensitivity of pharmacodynamics tests using prototype drugs such as neuroleptics, benzodiazepines, selective serotonin re-uptake inhibitors (SSRIs), ethanol, 3,4-methylenedioxy-methamphetamine (MDMA, or ecstasy), and cannabis [8–14]. With many such neuropharmacologically active substances, one or more parameters showed high sensitivity for a pharmacological effect in healthy subjects.

The characteristics of pharmacodynamics tests can play a crucial role in determining whether a drug produces an observable effect. Therefore, a pharmacodynamics test must be validated and a reference drug must show a consistent, reproducible effect at relevant (i.e., therapeutic) doses. In addition, a dose–response effect must be measured. Moreover, the test must be sensitive to the effects that are intended to be measured, and this is particularly important in the early stages of drug development, which typically involve relatively small numbers of healthy volunteers. Although measuring the PK/PD relationship can provide evidence of pharmacological action, this relationship can only be determined by repeatedly measuring the drug's effects while measuring its plasma concentration. Unfortunately, however, many CNS tests are time-consuming, are not adaptable, and/or have learning effects. Thus, in order to be suitable for our purposes, pharmacological biomarkers must be both brief and repeatable.

Studies that require repetitive use of a CNS test battery can be incorporated in the design of relatively simple studies and can include fundamental phase I objectives related to pharmacokinetics, safety, and tolerability. Moreover, domain-specific CNS effects and pharmacological endpoints can be incorporated easily into the study design. Importantly, CNS tests that are easy and relatively simply to perform interfere only minimally with other study requirements, allowing pharmacokinetics samples to be taken frequently and as needed while satisfying the need to monitor potential adverse events. Needless to say, studies that include pharmacodynamics testing in early drug development – particularly studies that investigate CNS effects – should have a double-blind, randomized, placebo-controlled design. Experience in recent decades has shown that adequate biomarkers can detect the effects of most drugs that affect CNS activity.

Some CNS drugs may not have significant and/or measurable effects in healthy subjects. Compounds that are designed to improve cognition are a good example of this issue, as improving cognition is extremely difficult in healthy subjects with relatively normal cognitive capability. In these cases, inducing a pharmacological challenge in healthy subjects may be used to reveal the drug's effects. For example, the muscarinic acetylcholine receptor antagonist scopolamine can reduce cognitive function in healthy subjects, thereby establishing a baseline for testing the effects of newly developed cognition-enhancing compounds [15].

On the other hand, an increasing number of compounds are designed to exert their effect on perturbed pharmacological systems without affecting the brain during normal function. In theory, such neuromodulatory drugs have a clear advantage: their activity is specific to the pathophysiological condition, and their effects – including any adverse effects – will resolve when the healthy physiological state returns. However, this neuromodulatory activity is difficult to detect in healthy subjects. For example, a single dose of a cannabinoid antagonist has no specific effect in healthy subjects, which can complicate the researcher's ability to predict pharmacologically active drug levels in early phases of development. In such cases, modulatory drug's that target perturb CNS function temporarily can be administered. In the above-mentioned case of cannabinoids, the cannabinoid system can be activated by administering a cannabinoid agonist such as tetrahydrocannabinol (THC), which produces effects that can be easily measured in healthy subjects. These effects can be suppressed with cannabinoid receptor antagonists in a concentration-related manner [16]. Another example includes using the serotonergic challenge to detect the effects of SSRIs [17] (which are also typically difficult to measure reliably [11,13]). Of course, these challenge tests are more complicated than performing test batteries of pharmacological biomarkers, and they require extensive validation. However, with adequate preparation, challenge tests can often provide compelling evidence of the pharmacological activity of neuromodulators and/or receptor antagonists and BBB penetration in early phases of development.

The NeuroCart

The NeuroCart CNS test battery contains the following core tests: saccadic eye movement, smooth pursuit eye movement, the Bowdle visual analog scale (VAS), the Bond and Lader VAS, body sway, adaptive tracking, visual verbal learning, and quantitative electroencephalography (qEEG) (Table 1).

Saccadic eye movements are extremely sensitive to a wide range of drugs that act as depressants on the CNS [18,19]. A distinct drug-sensitive eye movement system is smooth pursuit eye movements. This test has been validated at CHDR [20] and is based on the work of others [21].

A series of VASs as originally described by Norris [22] have been used previously to quantify the subjective effects of benzodiazepines [23]. From the original set of 15 scales, three composite factors – corresponding to alertness, mood, and calmness – were derived as described by Bond and Lader [24]. These factors are used to quantify the subjective effects of CNS drugs.

The body sway meter measures body movement in a single plane, thus providing a measure of postural stability. This method has been used extensively to demonstrate drug-induced postural instability [25].

Table 1. The tests included in the NeuroCart test battery and their related CNS domains [31].

NeuroCart test	Targeted function	Related CNS areas
Saccadic eye movement	Neurophysiologic function	Superior colliculus, substantia nigra, amygdala
Smooth pursuit	Neurophysiologic function	Midbrain
Adaptive tracking	Visuomotor coordination	Neocortex, basal nuclei, brain stem, cerebellum
Body sway	Balance	Cerebellum, brain stem
Visual verbal learning test (VVLT)	Memory	Hippocampus
VAS Bond and Lader	Alertness, mood, calmness	Cortex, prefrontal cortex
VAS Bowdle	Feeling high, internal and external perception	Cortex, prefrontal cortex, amygdala

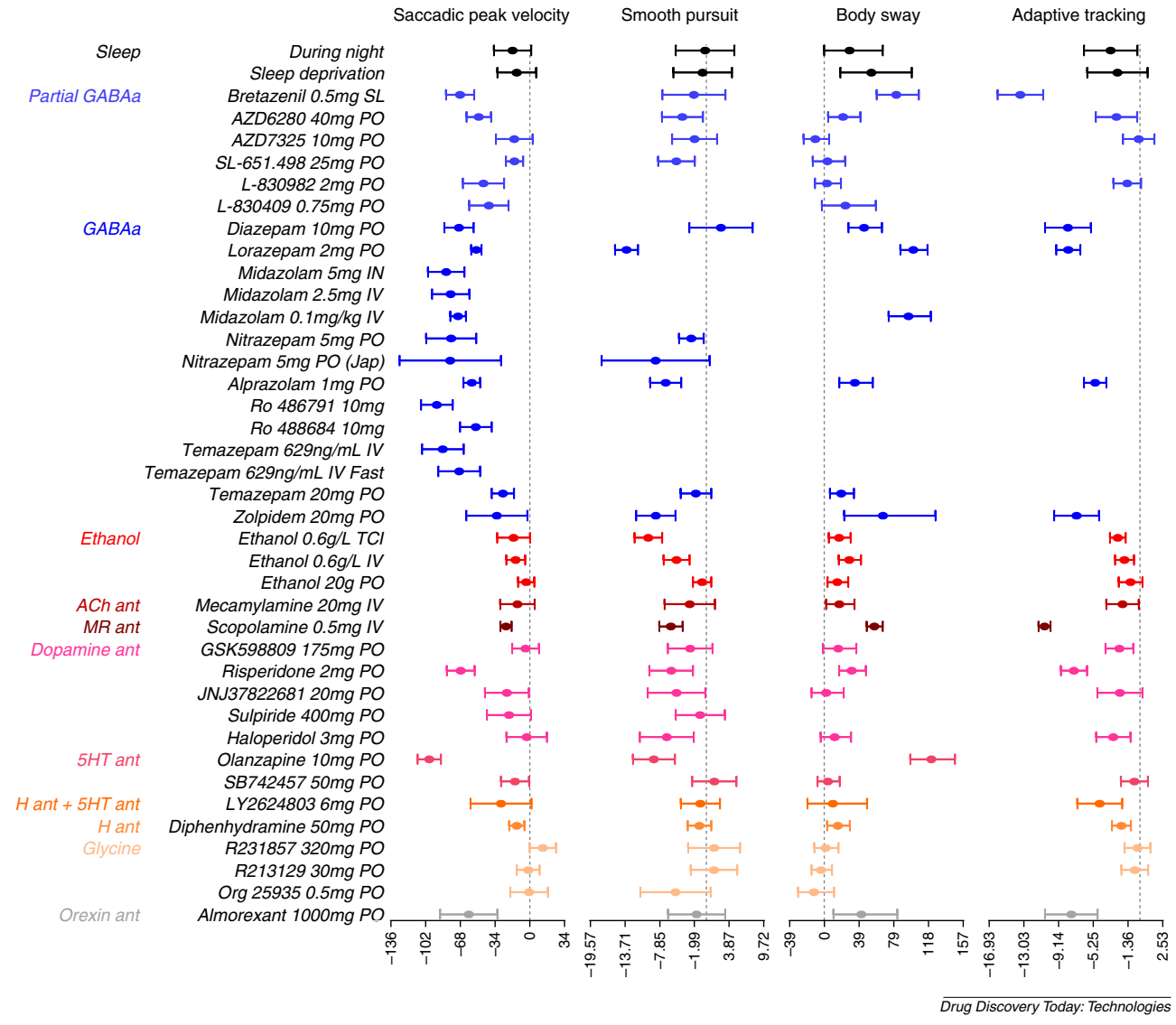

Figure 1. Forest plots summarizing the effects of depressant compounds on the indicated tests. On the *x*-axis is plotted the least square mean estimate of the difference from placebo (±95% CI) for saccadic peak velocity, smooth pursuit, body sway, and adaptive tracking. Each drug/compound is color-coded by drug class. The vertical dashed lines are reference lines used to assess significance and effect size.

The adaptive tracking test is based on specifications of Borland and Nicholson [26]. The adaptive tracking test provides a measure of visuomotor coordination and is extremely sensitive to a variety of psychoactive drugs and compounds that affect arousal and/or vigilance [18,25,27].

The visual verbal learning test measures various components of learning, including the acquisition, consolidation, storage, and retrieval of memories [28]. When administered to healthy subjects, the 30-word learning test does not have a ceiling effect, and it can reveal CNS effects induced by a variety of compounds, including benzodiazepines [29], cannabinoids [14], and antipsychotics [9].

EEG is one of the most widely used biomarkers in drug research, because of its preclinical and clinical applicability and its sensitivity to a wide range of conditions that affect CNS activity [30]. With the NeuroCart, EEG recordings are performed using silver chloride electrodes fixed with collodion adhesive at the Fz, Cz, Pz, and Oz positions; the same common ground electrode is used for the EEG recordings and the eye movement measurements.

Profiling CNS drugs using the NeuroCart

As discussed above, tests that measure the pharmacodynamics effects of drugs on CNS functions can be used to provide evidence that the drug has crossed the BBB and entered the brain. In particular, establishing a dose-effect relationship can provide compelling evidence that the test compound's effect is in the CNS (and – by extension – that the test compound has penetrated the BBB). The

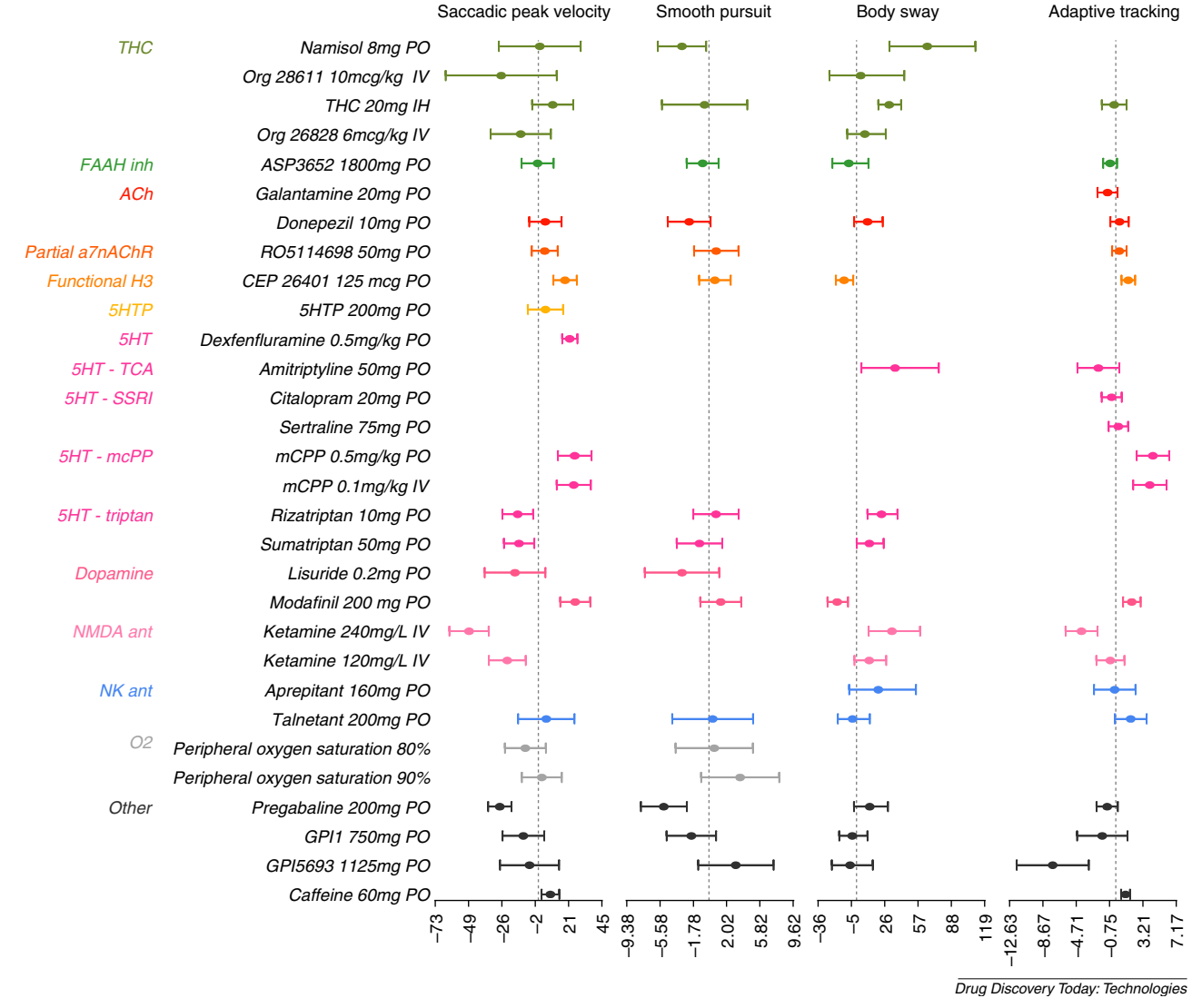

Figure 2. Forest plots summarizing the effects of depressants, antidepressants, and stimulants on saccadic peak velocity, smooth pursuit, body sway, and adaptive tracking. See Fig. 1 for details.

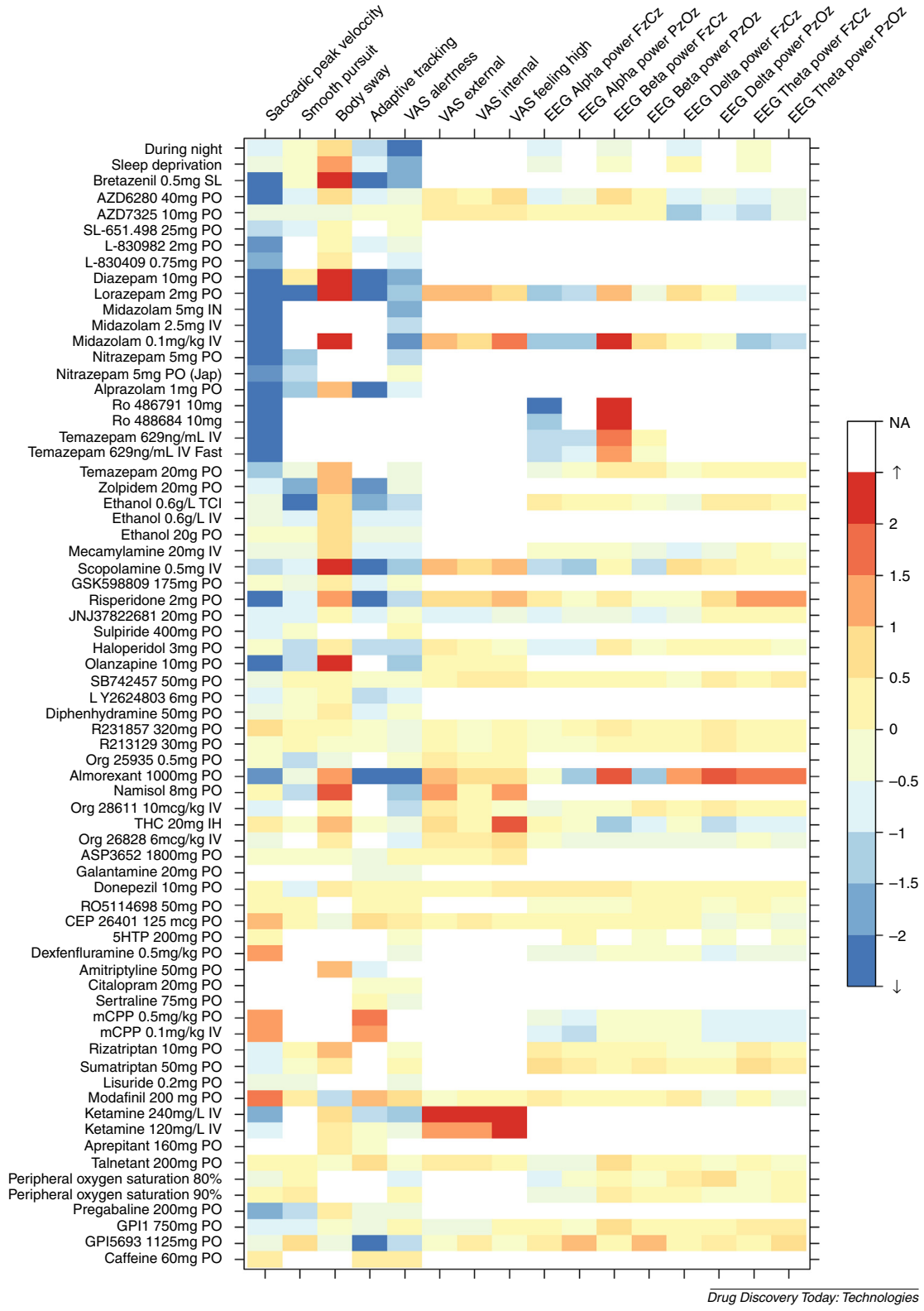

Figure 3. Heat plot depicting the ratios between the measured drug effects and the 'minimal detectable effect sizes'. 'Warmer' colors indicate an increase, and 'colder' colors indicate a decrease of activity. Data that are not available (NA) are plotted in white.

NeuroCart is a multi-dimension CNS test battery that was created using information obtained from an extensive literature reviews [8–14]. An important advantage of the approach used to develop the NeuroCart is that it led to a core battery of tests that have not changed significantly over the years, allowing the creation of a relatively static panel of cognitive, psychomotor, subjective drug effect, and neurophysiological measurements measured using a wide variety of drugs and drug classes. Using this comprehensive panel, researchers can define profiles of drug classes – or even individual drugs – with respect to their effect on various NeuroCart tests. In a recent meta-analysis, we analyzed the pharmacodynamics profiles of a range of compounds, including antidepressants, stimulants, and CNS depressant agents; the data were obtained from 38 studies that used various CNS active drugs. The results of this meta-analysis are summarized in Figs 1 and 2. Specifically, the effect profiles of both the registered drugs and the compounds under development are shown for four NeuroCart tests; these tests were chosen because they were the most informative with respect to the stimulant and depressant properties of CNS active compounds. Figure 3 shows the effects of the same 38 compounds measured using all of NeuroCart's tests, displayed as a heat plot. This type of plot can also be used to visually determine the pharmacodynamics profile for a new CNS active compound. A direct comparison between two pharmacologically related compounds can also be displayed as a spider web plot, clearly illustrating the differences in profiles between compounds, as well as the dose-dependent effects of the drug being developed.

In summary, clear evidence showing that a compound can penetrate the BBB can be obtained using the NeuroCart test battery. Moreover, the NeuroCart can use to create a unique 'fingerprint' (profile) with respect to both desired and undesired CNS effects. This versatile research tool can be included in any phase I drug study, which typically covers the widest range of doses and concentrations in the entire drug development program. Consequently, for drugs with established modes of action, the NeuroCart can already provide indications for pharmacological activity, therapeutically relevant concentration ranges, and BBB-penetration, during the first administration in man [32,33]. Equally importantly, the absence of CNS-effects for these compounds is a certain sign that the drug did not reach the brain. For compounds with novel mechanisms of action, demonstration of concentration-dependent CNS-effects will provide similar confidence that the drug penetrated the brain. However, the absence of such effects may be related to limitations of the NeuroCart to detect the novel mechanism, and further studies with repeated dosing or other methods (CSF-sampling, PET) may still be warranted. With these considerations, the proper use of CNS-measurements in early drug development can provide important information that can be used to make a go/no-go decision regarding further development, as well as to guide the decision-making process regarding the dosage range to be used in phase II studies, determining a therapeutic window, and even identifying the target population.

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